Multiple Mini-Format 2-D Electrophoresis

SE 600 Standard Vertical Unit

Keywords: 2-D Electrophoresis, 7 cm IPG Drystrip

Two-dimensional (2-D) electrophoresis is a powerful and widely used method for the analysis of complex protein mixtures. With the recent improvements to and uses for this technique, the need for higher throughput has increased. This application note describes a method for performing the second-dimension separation for two to eight mini-format 2-D gels simultaneously in a single vertical electrophoresis unit. The procedure yields high-quality separations, using 7 cm Immobiline™ DryStrip IPG gel strips and the SE 600 Dual Vertical Gel unit. This arrangement simplifies and improves differential comparisons. Using a third glass plate and additional spacers for the SE 600, a "club sandwich" assembly can be formed which produces two identical gels in a single gel space. Thus eight second-dimension separations can be performed on one unit: two IPG strips per gel, two gels per sandwich, and two sandwiches per unit. At approximately three hours per run, 16 mini-format 2-D separations can be completed in a normal workday on a single SE 600 system.

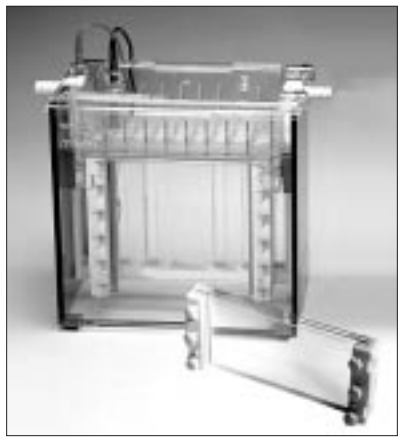

Fig 1 SE 600 and clamp assembly

Protocol

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Casting gels

1.1 Assemble the SE 600 vertical slab gel unit in the dual gel casting stand using 18 × 8 cm glass plates (80-6186-59). For running eight 2-D mini-gels simultaneously, use the glass plate club sandwich dividers (80-6186-78). The divider plates allow two gels to be cast in a single sandwich. Be sure to use the 8 cm × 1 cm × 1 mm (80-6443-09) or 8 cm × 1 cm × 1.5 mm (80-6443-28) spacers.

1.2 Cast the gels following the instructions provided in the SE 600 Series User Manual (80-6353-79) or *2-D Electrophoresis Using Immobilized pH Gradients: Principles & Methods* (80-6429-60, "2-D Principles handbook"). Appropriate volumes of acrylamide are listed in Table 1.

Products used

SE 600 Dual Cooled Vertical Unit	80-6171-58
Glass plates, 18 × 8 cm (2/pk)	80-6186-59
Glass plate, club sandwich divider, notched, 18 × 8 cm	80-6186-78
Clamp assembly, 8 cm (2/pk)	80-6187-35
Spacer, 1.0 mm, 1 cm wide, 8 cm long (2/pk)	80-6443-09
Spacer, 1.5 mm, 1 cm wide, 8 cm long (2/pk)	80-6443-28
EPS 601 Power Supply	18-1130-02
Plastic ruler	80-6223-83
MW markers, 2 512–16 949 range	80-1129-83
MW markers, 14 400–94 000 range	17-0446-01
IEF Sample Application Pieces (200/pk)	80-1129-46

PlusOne™ Reagents

Urea, 500 g	17-1319-01
Tris, 500 g	17-1321-01
SDS, 100 g	17-1313-01
Glycerol (87%), 1 litre	17-1325-01
Bromophenol blue	17-1329-01
Glycine, 500 g	17-1323-01
Agarose M, 10 g	17-0422-01
Agarose NA, 10 g	17-0554-01
Dithiothreitol (DTT), 1 g	17-1318-01
Acrylamide IEF 40% solution, 1 000 ml	17-1301-01
N,N'-methylenebisacrylamide, 25 g	17-1304-01
Ammonium persulphate, 25 g	17-1311-01

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Equilibrate the IPG strip

IPG strips are run and equilibrated for application to the second dimension as described in the 2-D Principles handbook. Soak strips for 15 min in equilibration solution (solution A) containing 1% (w/v) DTT followed by 15 min more in equilibration solution containing 2.5% (w/v) iodoacetamide. Two 7-cm-long IPG strips can be equilibrated simultaneously in a 15 ml conical centrifuge tube using 6 ml of equilibration solution.

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Apply the equilibrated IPG strip

3.1 Place the IPG strip.

Dip the IPG strip in the SDS electrophoresis buffer (solution B) to lubricate it. Position the first IPG strip between the plates on the surface of the second-dimension gel with the plastic backing against one of the glass plates. Be sure to place the strip as far as possible to one side of the gel. With a thin plastic ruler (80-6223-83), gently push the IPG strip down so its entire lower edge is in contact with the top surface of the slab gel. Ensure that no air bubbles are trapped between the IPG strip and the slab gel surface or between the gel backing and the glass plate. Repeat this procedure, placing the second strip next to the first and leaving a space of approximately 1 cm between the strips.

3.2 Optional: Apply molecular weight marker proteins.

The markers are applied in 0.5% agarose (M or NA) to a paper IEF sample application piece in a volume of 15 to 20 µl. For less volume cut the sample application piece proportionally. Add 0.5% agarose to the appropriate volume of marker solution and heat at 100 °C for approximately 5 min. Place the IEF application piece on a glass plate and pipette the marker solution onto it, then pick up the application piece with forceps and apply to the top surface of the gel in the space between the two IPG strips. The markers should contain 200 to 1 000 ng of each component for Coomassie staining and about 10 to 50 ng of each component for silver staining.

3.3 Seal the IPG strip in place.

Embedding the IPG strips in agarose prevents them from moving or floating in the electrophoresis buffer. Prepare agarose sealing solution (solution C). Melt each aliquot as needed in a 100 °C heat block (each gel will require 0.5 to 1 ml). It takes approximately 10 min to fully melt the agarose. (*Tip:* An ideal time to prepare and melt the agarose is during the IPG strip equilibration-step 2.) Allow the agarose to cool to 40 to 50 °C and then slowly pipette onto the top of the gel the amount required to seal the IPG strip in place. Pipetting slowly avoids introducing bubbles. Allow a minimum of 1 min for the agarose to cool and solidify.

3.4 Finish assembling the electrophoresis unit (see the SE 600 Series User Manual 80-6353-79) and add SDS electrophoresis buffer (solution B). This method will require a total of 5 litres of buffer (4.5 litres for the anode and 0.5 litre for the cathode).

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Electrophoresis conditions

Electrophoresis is performed at constant current in two steps. Start at 15 mA/gel for an initial migration and stacking period of 15 min, then increase to 30 mA/gel for a period of approximately 3 h. Currents up to 50% higher may be used during the second separation phase if only two gels per unit are being run (no club sandwich dividers) and the unit is being cooled with a thermostatic circulator. Stop the electrophoresis when the dye front is approximately 1 mm from the bottom of the gel.

Cooling is optional; however, temperature control improves gel-to-gel reproducibility, especially if the ambient temperature of the laboratory fluctuates significantly. Do not cool SDS-containing gels below 15 °C.

After electrophoresis remove gels from their cassettes in preparation for staining or blotting. Notch or mark each gel at the upper corner nearest the pointed end of the IPG strip to identify the acidic end of the first-dimension separation.

Conclusions

This multiple mini-format method produces high-quality 2-D separations (Fig 2) and allows for two to eight mini-format 2-D gels to be run on a single SE 600 unit. Differential comparisons are improved by this method by minimizing gel-to-gel variations. In addition, this method allows for increased throughput with minimal equipment. At approximately three hours per second-dimension run, 16 mini-format 2-D separations can be run in a normal workday on a single SE 600 system.

For more-detailed information and troubleshooting of 2-D electrophoresis, see the 2-D Principles handbook.

Fig 2 Multiple Mini-Format 2-D Gel. E. coli extract (24 µg), pH 3-10 NL first dimension; 1-mm thick 12.5% acrylamide second dimension

Table 1. Volumes of Acrylamide Required for Mini-Format 2-D Gels

	Total Volume (ml)	
Number of 16 × 8 cm Gels	**2**	**4**
1.5-mm-thick spacers	40	80
1-mm-thick spacers	30	60

Solutions required

A. SDS equilibration buffer[1]

(50 mM Tris-Cl pH 8.8, 6M urea, 30% glycerol, 2% SDS, bromophenol blue, 200 ml)

	Final concentration	Amount
1.5 M Tris-Cl, pH 8.8 (solution D)	50 mM	6.7 ml
Urea (FW 60.06)	6 M	72.02 g
Glycerol (87% v/v)	30% (v/v)	69 ml
SDS (FW 288.38)	2% (w/v)	4 g
Bromophenol blue	trace	(a few grains)
Double-distilled H_2O		to 200 ml

[1] This is a stock solution. Prior to use, DTT or iodoacetamide is added. See section 2. Store in 40 ml aliquots at –20 °C.

B. SDS electrophoresis buffer[1]

(25 mM Tris, 192 mM glycine, 0.1% SDS, 5 litres)

	Final concentration	Amount
Tris base (FW 121.1)	25 mM	15.1 g
Glycine (FW 75.07)	192 mM	72.1 g
SDS (FW 288.38)	0.1% (w/v)	5.0 g
Double distilled H_2O		to 5 000 ml

[1] Because you do not need to titrate this buffer to a specific pH, it can be made up directly in large reagent bottles marked at 5 litres. 20 litres can be made up at a time. Store at room temperature.

(continued on page 4)

Solutions required (continued)

C. Agarose sealing solution

	Final concentration	Amount
SDS electrophoresis buffer (solution B)		100 ml
Agarose (NA or M)	0.5%	0.5 g
Bromophenol blue	trace	a few grains

Add all ingredients into a 500 ml Erlenmeyer flask. Swirl to disperse. Heat in a microwave oven on low until the agarose is completely dissolved. Do not allow the solution to boil over. Dispense 2 ml aliquots into screw-cap tubes and store at room temperature.

D. 1.5 M Tris-Cl, pH 8.8

	Final concentration	Amount
Tris base (FW 121.1)	1.5 M	181.5 g
Double-distilled H_2O		750 ml
HCl (FW 36.46)		adjust to pH 8.8
Double-distilled H_2O		to 1 000 ml

Filter solution through a 0.45 µm filter. Store at 4 °C.

For more information, visit the Amersham Phamacia Biotech Website: www.amershambiosciences.com

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